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Influence of the epithelium on responsiveness of guinea-pig isolated trachea to contractile and relaxant agonists

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1 The potency (pD_2) and maximal contractile effect (E_{max}) of histamine, acetylcholine, carbachol and K^+ were assessed from cumulative concentration-effect curves in guinea-pig isolated tracheal ring preparations with and without an intact epithelium.

2 Estimates of E_{max} were not significantly different in epithelium-denuded preparations compared with those measured in intact preparations; pD_2 values for acetylcholine, carbachol and K^+ were not significantly altered. In contrast, the potency of histamine was significantly increased by about 4 fold in preparations devoid of epithelial cells.

3 Estimates of potency and E_{max} were also determined for the smooth muscle relaxants isoprenaline, forskolin and theophylline (which increase intracellular cyclic AMP) and for nitroglycerin (which increases cyclic GMP) in both intact and epithelium-stripped tracheal rings. The pD_2 values for these relaxants were not significantly altered by the removal of the epithelium. However, with the exception of nitroglycerin, E_{max} values for these relaxants were significantly lower in stripped than in intact tracheal rings that had been maximally precontracted with carbachol.

4 The autoradiographic localisation of binding sites for the non-selective β-adrenoceptor ligand [^{125}I]-iodocyanopindolol (I-CYP) showed that the epithelium of the guinea-pig trachea had a 75 ± 16% greater density of β-adrenoceptors than the smooth muscle. Removing the epithelium did not significantly alter either the density of smooth muscle binding sites or the affinity of I-CYP binding. It was concluded that the reduced functional response of guinea-pig trachea to isoprenaline was probably not due to smooth muscle β-adrenoceptor dysfunction.

5 Results indicate that the epithelium plays an important role in the modulation of responsiveness of guinea-pig trachea to histamine and relaxants that mediate their effects by selectively increasing intracellular cyclic AMP levels.

Introduction

It is well established that acetylcholine(ACh)-induced relaxation of isolated artery preparations is dependent on the presence of an intact endothelium (Furchgott & Zawadzki, 1980; Chand & Altura, 1981a; De Mey *et al.*, 1982). Relaxation seems to be mediated via the production of an endothelium-derived relaxing factor (EDRF) formed as an unstable oxidation product of arachidonic acid possibly via the lipoxygenase pathway (Furchgott & Zawadzki, 1980; Chand & Altura, 1981b; Singer & Peach, 1983; Föstermann & Neufang, 1984 a,b). Removal of vascular endothelial cells results in ACh causing no response or contraction of isolated vascular preparations (Furchgott & Zawadzki, 1980; Diamond & Chu, 1983). Similar changes in the responsiveness of guinea-pig pulmonary artery to histamine have been demonstrated (Satoh & Inui, 1984).

The purpose of the present study was to examine the possibility that the epithelium of isolated airways preparations also produce a relaxing factor in response to contractile agonists, which is capable of modulating their spasmogenic potency or maximal effect. The influence of the airways epithelium on responsiveness to the relaxant agonists isoprenaline, forskolin, theophylline and nitroglycerin was also examined.

Methods

Organ bath studies

Guinea-pigs (SR/C Tricolour) of either sex weighing 500–600 g were stunned by a blow to the head and exsanguinated. The trachea was removed and cut into rings approximately 2 mm in width. Adjacent pairs of tracheal rings served as control (epithelium intact) and test (epithelium removed).

The epithelium was removed from all test preparations by gently rubbing their mucosal surfaces with a cotton wool coated probe. All preparations were suspended under 500 mg tension in Krebs-Henseleit solution maintained at 37°C and aerated with 5% CO_2 and 95% O_2. The composition of Krebs-Henseleit solution was (mM): NaCl 117.6, KCl 5.4, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.57, D-glucose 11.1 and $CaCl_2$ 2.5. Particular care was taken to ensure that as little damage as possible was caused to the epithelial layer of control ring preparations by the suspensory threads in the organ bath. Changes in isometric tension were monitored with a Grass force-displacement transducer (FTO3C) coupled to a preamplifier and Rikadenki pen recorder (model 1328L). Preparations were left to equilibrate for 60–90 min with regular washing by gravity drainage and refilling before drug-induced responses were measured. It was usual for tracheal rings to gain some tone spontaneously. In a few cases small decreases in resting tension occurred and these were offset by readjustment to 500 mg. All preparations were then exposed to single priming concentrations of carbachol (0.8–0.2 μM), as previously described (Goldie *et al.*, 1982), before a cumulative concentration-effect curve to either carbachol, acetylcholine, histamine or potassium ions (K^+) was constructed. After washout and a rest period of 45 min, a second cumulative concentration-effect curve to the same spasmogen was constructed in some preparations. Estimates of potency ($pD_2 = -\log_{10}$ molar EC_{50}) and maximal contractile effect (E_{max}) were determined from these curves. E_{max} was calculated as the maximum increase in tone (mg).

In some experiments, the effects of the histamine H_2-receptor antagonist cimetidine (10 and 50 μM) on the potency and maximal contractile response to histamine were examined. Control cumulative concentration-effect curves to histamine were constructed in intact and epithelium-denuded tracheal rings. Further curves were constructed in the presence of cimetidine. As for all test agonists, control curves to histamine were produced in some preparations to estimate time-related changes in potency and E_{max}.

Other preparations were precontracted with EC_{50} concentrations of carbachol and consecutive cumulative concentration-effect curves to the relaxant agonists isoprenaline, theophylline, forskolin or nitroglycerin constructed. Both EC_{50} and E_{max} values were determined for these relaxant agonists. E_{max} was calculated as the maximal decrease in tone (mg). All preparations were then fixed for 24 h in buffered formal saline (pH 7.0) embedded in paraffin and sectioned at 6 μm. Sections were stained with haematoxylin and eosin and the integrity of the airway epithelium assessed at the light microscopic level.

Autoradiography

Segments of guinea-pig trachea approximately 4 mm in length, with the epithelium intact or removed, were placed side by side in aluminium foil pans containing OCT embedding medium, and snap frozen in isopentane, quenched with liquid nitrogen. Serial frozen sections of tracheal tissue (10 μm, autoradiographs; 16 μm, kinetic experiments) from both intact and epithelium-denuded preparations were cut at −30°C and mounted and thawed onto washed, gelatinized glass slides as described by Young & Kuhar (1979). Sections were incubated at 22°C for 150 min in Tris-HCl buffer (170 mM; pH7.6) containing [^{125}I]-iodocyanopindolol (I-CYP, 50 pM autoradiographs; 10–320 pM, kinetic experiments) and the protease inhibitor PMSF (10 μM) according to the methods of Summers *et al.* (1985). Non-specific binding was estimated by the addition of isoprenaline (200 μM) to the incubation solution. All sections were then rapidly rinsed in Tris-HCl buffer (170 mM, pH 7.6) followed by two 15 min washes in buffer and a final rinse in distilled water. Sections for autoradiography were then rapidly dried under a stream of cold dry air, while those for binding analysis were wiped from slides onto GF/A glass fibre filter paper and radioactivity levels estimated using a Packard gamma counter (Model 5650). In the kinetic experiments, estimates of binding constants were obtained using Scatchard and Hill analyses as well as the non-linear iterative curve fitting program NONLIN (Metzler, 1969). Non-linear analyses were performed using weighted data (I/Y observed; Peck *et al.*, 1984).

Coverslips (type 0) were coated by dipping into NTB-3 photographic emulsion (Eastman Kodak Co., Rochester, N.Y.) diluted 1:1 with distilled water, at 46°C, air dried for 3 h and stored over dessicant. Emulsion coated coverslips were attached to one end of tissue slides in the dark with cyanoacrylate adhesive and placed in light-tight X-ray cassettes at 4°C for 3 days. The emulsion was developed (for 3 min) in Dektol (Kodak) diluted 1:1 with distilled water and fixed (for 3 min) with Rapidfix (Kodak) diluted 1:4 with distilled water containing 2.5% Hypam hardener (Ilford). The tissue was lightly stained with 0.05% toluidine blue in 0.5% borax for 30 s. After air drying, the coverslips were re-apposed to the slides and the tissue mounted in DePeX medium (BDH). Sections

were viewed with a Leitz Ortholux II photomicroscope under light and dark field illumination.

Quantitation of I-CYP binding sites was achieved by manual counting of autoradiographic grains visualised on dark-field photomicrographs of fields described within areas of tracheal epithelium and smooth muscle. One hundred and seventy fields each with an area of approximately 4500 μm^2 were chosen within tissue sections labelled with I-CYP in the absence and presence of 200 μM isoprenaline. For intact preparations, a total tissue area of $1.9-2.4 \times 10^5$ μm^2 was analysed with respect to grain density over both tracheal epithelium and smooth muscle for the estimation of total and non-specific binding. A similar area over smooth muscle was examined in epithelium-denuded preparations.

The probability of differences between mean values for parameters measured in both organ bath and autoradiographic studies was determined by Student's two-tailed, non-paired t test and considered significant if $P < 0.05$.

Drugs

Drugs used were: acetylcholine chloride, carbamylcholine chloride, histamine diphoshate, (−)-isoprenaline hydrochloride, theophylline (Sigma); potassium chloride (analytical grade, B.D.H.); forskolin, phenylmethylsulphonylfluoride (PMSF; Calbiochem), nitroglycerin (Fisons), cimetidine hydrochloride (S.K.F); (−)-[^{125}I]-iodocyanopindolol (Amersham). All drugs tested in organ bath studies were prepared daily in 0.9% w/v NaCl solution (saline). Isoprenaline was prepared in saline containing 20 $\mu g\ ml^{-1}$ ascorbic acid.

Results

Light microscopic examination of paraffin sections of tracheal rings revealed the presence of a pseudostratified columnar ciliated epithelium. Several different mechanical methods of removing the epithelial cell layer from guinea-pig tracheal rings were initially tested. These included rubbing the walls of the ring preparations with a narrow wooden or plastic probe, a length of silver wire or the coarse bristles of a small brush. Light microscopic examination of the treated rings showed that many of these methods failed to remove the epithelium completely and sometimes caused damage to underlying tissues including the smooth muscle. However, the use of a cotton wool coated probe was found consistently to cause complete removal of epithelium without damaging other tissue structures (Figure 1).

Contractile agonists

Acetylcholine (ACh), carbachol (CCh), histamine and K^+ caused concentration-dependent contractions of both intact and epithelium-denuded guinea-pig airway preparations. Table 1 shows that the order of potency for these spasmogens was CCh > ACh > histamine > K^+, as determined from first cumulative concentration-effect curves in both control (1:4.6:100:181000) and epithelium-denuded (1:4.3:35.5:214000) guinea-pig tracheal rings. There was no significant difference in the potency of CCh, ACh or K^+ in intact preparations compared with that in epithelium-denuded tracheal rings (Table 1). Conversely, the potency of histamine as assessed from first curves, was significantly enhanced by 3.8 fold ($P < 0.001$). However, the increase in the potency of histamine in these preparations was only 2.4 fold ($P < 0.01$) when this was determined from second curves. A small but significant increase in the potency of CCh in stripped preparations was also detected from second curves. All 4 spasmogens produced similar maximal increases in tracheal tone. The sizes of maximal contractile responses to these spasmogens were not significantly different in intact and epithelium-denuded preparations (Figure 2).

Cimetidine Cimetidine failed to enhance the contractile potency of histamine in either intact or stripped tracheal rings. Thus, in the presence of 10 μM and 50 μM cimetidine, histamine was still 2.0 and 2.9 times more potent respectively in stripped than in intact preparations. Similarly, cimetidine (10 and 50 μM) did not significantly alter E_{max} values for histamine in these intact and stripped tracheal rings ($P > 0.2$).

Relaxant agonists

Isoprenaline, forskolin, theophylline and nitroglycerin produced a concentration-dependent relaxation of both intact and epithelium-denuded, CCh-contracted guinea-pig tracheal rings. Table 2 shows that the potency order for relaxation was isoprenaline > forskolin > nitroglycerin > theophylline in both types of tracheal ring preparation. The potencies of these relaxants relative to isoprenaline were 1:61:224:33,000 in intact preparations and 1:55:239:42,000 in stripped tracheal rings. There was no significant difference in the potency of isoprenaline forskolin, nitroglycerin or theophylline in intact preparations compared with that in stripped rings (Table 2). Mean maximal relaxant responses to isoprenaline, forskolin, nitroglycerin and theophylline, in tracheal rings submaximally precontracted with CCh (EC_{50}) were 33%, 26%, 8% and 27% smaller respectively in epithelium-denuded preparations than in intact preparations. However, none of

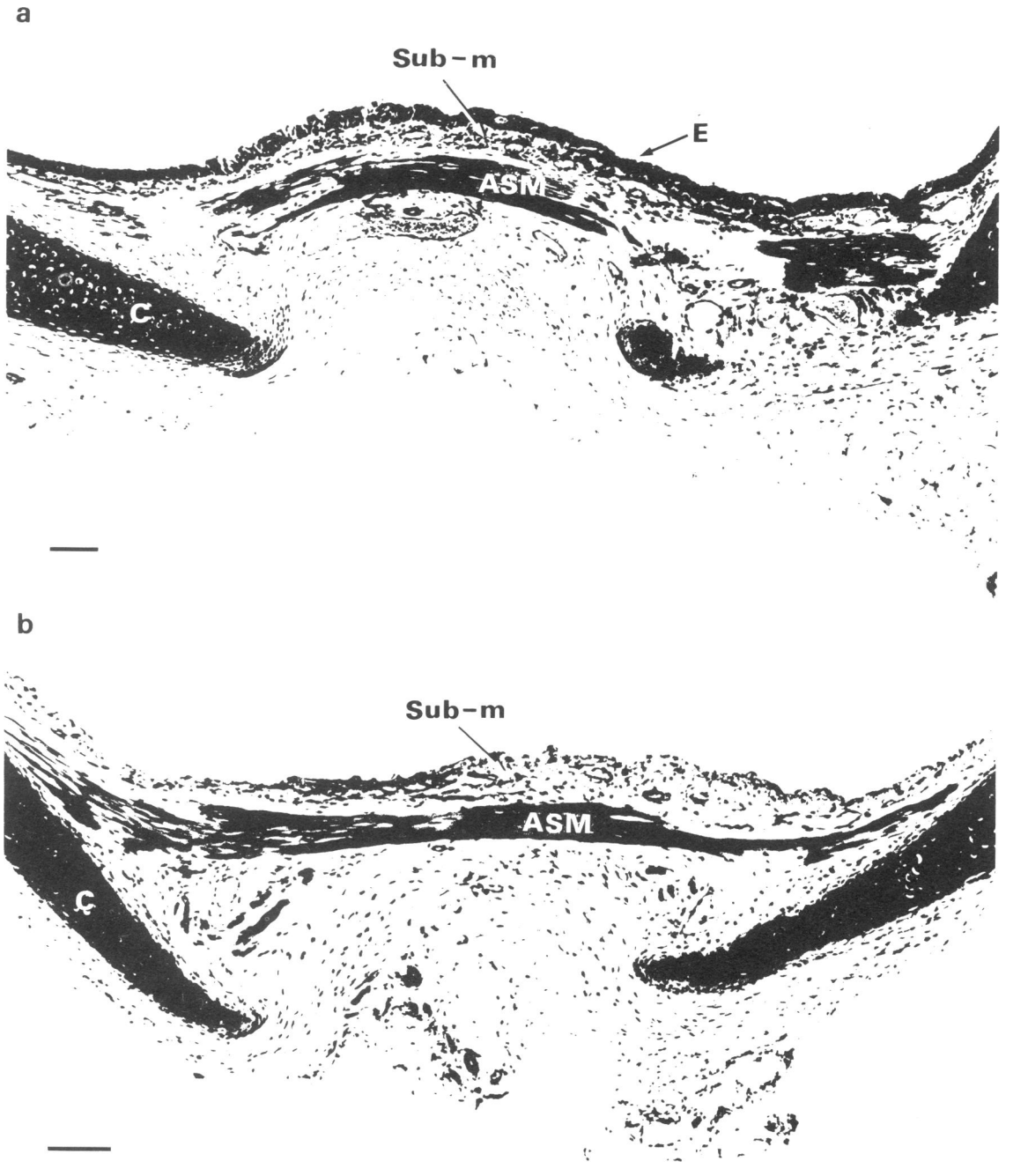

Figure 1 Transverse paraffin embedded sections of guinea-pig trachea cut at 6 μm and stained with haematoxylin and eosin. (a) Epithelium (E) intact. (b) Epithelium removed. ASM = airway smooth muscle; Sub-m = submucosa; C = cartilage. Bar = 100 μm.

Table 1 Effect of epithelium stripping on the potency (pD_2) of contractile agonists in guinea-pig tracheal rings

	1st curve			*2nd curve*		
	Control		*Stripped*	*Control*		*Stripped*
Histamine						
pD_2‡	5.16 ± 0.05 (28)		5.74 ± 0.07 (27)	5.25 ± 0.06 (11)		5.63 ± 0.05 (5)
*P**		<0.001			<0.01	
Ratio†		3.80			2.40	
Acetylcholine						
pD_2‡	6.50 ± 0.08 (12)		6.66 ± 0.12 (12)	6.32 ± 0.14 (6)		6.36 ± 0.22 (6)
*P**		>0.1			>0.1	
Ratio†		1.44			1.10	
Carbachol						
pD_2‡	7.16 ± 0.06 (32)		7.29 ± 0.05 (34)	6.86 ± 0.05 (11)		7.11 ± 0.03 (11)
*P**		>0.1			<0.001	
Ratio†		1.35			1.78	
Potassium						
pD_2‡	1.90 ± 0.05 (10)		1.96 ± 0.03 (10)			
*P**		>0.1				
Ratio†		1.14				

Data are presented as mean ± s.e.mean.
Numbers in parentheses indicate the number of observations.
‡pD_2 values for each drug in control and stripped preparations were calculated as = $-\log_{10}(EC_{50})$.
†Ratio of EC_{50} values (derived from mean pD_2 values) = EC_{50} control; EC_{50} stripped.
*P = the probability of differences between mean pD_2 values determined using Student's non-paired t test, and considered significant if $P<0.05$.

these apparent reductions reached statistical significance ($P>0.05$).

Spontaneously developed tone was usually at least as large a component of total tracheal tone as was that induced by CCh, but this varied greatly between preparations as well as from curve to curve within preparations. Isoprenaline, forskolin and theophylline produced similar mean decreases in control tracheal tone i.e. 200–250% of CCh-induced tone. In contrast, nitroglycerin had considerably lower intrinsic activity than each of the other relaxants, such that the maximal relaxation was 97–110% of CCh-induced tone. Thus spontaneous tracheal tone did not complicate responsiveness to nitroglycerin.

In tracheal rings maximally precontracted with CCh ($4 \times EC_{90}$), in an attempt to minimize the variable contribution of spontaneous tone to the final level of tone, isoprenaline produced a 33% smaller ($P<0.02$) mean maximal relaxation in stripped than in intact preparations (Figure 3). Control isoprenaline potency was approximately 40 fold lower in these rings than in those with submaximal CCh-induced tone. However, the potency of isoprenaline was not significantly different in intact and epithelium-denuded preparations ($P>0.1$). Furthermore, mean levels of CCh-

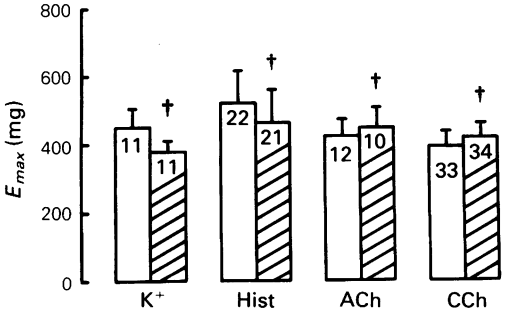

Figure 2 Comparison of mean maximal contractile effects (E_{max}, mg) to potassium (K^+), histamine (Hist), acetylcholine (ACh) and carbachol (CCh) in guinea-pig tracheal ring preparations with an intact epithelium (open columns) and in preparations denuded of epithelium (hatched columns). Numbers in columns indicate the number of separate observations made. Vertical bars on columns represent s.e.mean. †Not significantly different cf. mean in preparations with an intact epithelium ($P>0.2$, Student's non-paired t test).

Table 2 Effect of epithelium stripping on the relaxant potencies of isoprenaline, forskolin, theophylline and nitroglycerin in guinea-pig tracheal rings

	Isoprenaline	*Forskolin*	*Nitroglycerin*	*Theophylline*
Control				
pD_2‡	8.48 ± 0.05	6.70 ± 0.03	6.13 ± 0.06	3.95 ± 0.05
	(11)	(6)	(10)	(12)
EC_{50} (M)*	3.3×10^{-9}	2.0×10^{-7}	7.4×10^{-7}	1.1×10^{-4}
Stripped				
pD_2‡	8.51 ± 0.05†	6.77 ± 0.03†	6.13 ± 0.06†	3.90 ± 0.03†
	(11)	(6)	(11)	(12)
EC_{50} (M)*	3.1×10^{-9}	1.7×10^{-7}	7.4×10^{-7}	1.3×10^{-4}

All preparations were pre-contracted with that concentration of carbachol producing 50% of the maximal response to this spasmogen. Results were obtained from second relaxant cumulative concentration-effect curves. Data were presented as mean ± s.e.mean. Numbers in parentheses indicate the number of observations.
‡pD_2 values for each drug in control and stripped preparations were calculated as $= -\log_{10}(EC_{50})$. †Not significantly different cf. respective control value ($P > 0.2$; Student's non-paired t test). *Derived from mean pD_2 value.

induced tone were again not significantly different in intact and stripped tracheal rings ($P > 0.2$). Similar results were obtained when theophylline and forskolin were used as relaxants. Theophylline produced a 36% smaller mean maximal relaxation in stripped than in intact preparations ($P < 0.02$), while the relaxations to forskolin were on average 20% smaller ($P < 0.05$). Conversely, the potencies of these relaxants were not significantly different in the two types of airway preparation ($P > 0.2$). Neither the potency nor the maximal relaxant response to nitroglycerin was significantly altered by removing the tracheal epithelium.

Kinetic experiments

The specific binding of I-CYP in sections from both intact and epithelium-denuded guinea-pig trachea was saturable. Hill coefficients (nH) approached unity indicating specific binding to a single class of sites with high affinity in both unstripped and epithelium-stripped preparations (dissociation constant K_D = 74–75 pM, 69–80 pM respectively; Table 3).

Non-specific binding accounted for approximately 31% and 46% of total binding at a concentration of I-CYP of 75 pM in intact and epithelium-denuded trachea, respectively. Removing the epithelium caused a reduction in the maximum binding capacity (B_{max}), of tracheal sections, of approximately 50% (Figure 4, Table 3).

Autoradiography

The distribution of autoradiographic grains in sections of intact and epithelium-denuded guinea-pig trachea is shown in Figure 5. Sections incubated with both I-CYP and isoprenaline (200 μM) did not show a specific labelling pattern. Thus they indicate the distribution of non-specific binding sites for I-CYP sites for I-CYP in the trachea were heavily concentrated in the epithelial cell layer and in the mucosal cell layers immediately below the epithelium. The density of specific I-CYP binding sites in the tracheal epithelium and mucosal cell layers was 75 ± 16% higher than that observed in the smooth muscle band, as determined from the means of results from 3 separate

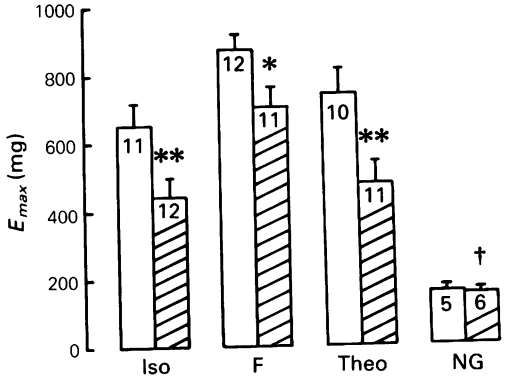

Figure 3 Comparison of mean maximal relaxant effects (E_{max}, mg) to isoprenaline (Iso), forskolin (F), theophylline (Theo) and nitroglycerin (NG) in guinea-pig tracheal ring preparations with an intact epithelium (open columns) and in preparations denuded of epithelium (hatched columns). All preparations were precontracted with a 4 fold higher concentration of carbachol than that which produced 90% of the maximal contraction to this spasmogen. Numbers in columns indicate the number of separate observations made. Vertical bars on columns represent s.e.mean. Significantly smaller maximal relaxant response cf. that in preparations with in intact epithelium is indicated as follows, *$P < 0.05$; **$P < 0.02$; †$P > 0.2$ (Student's non-paired t test).

Table 3 Estimates of parameters describing specific binding of [^{125}]-iodocyanopindolol (I-CYP) in sections of guinea-pig trachea with an intact (control) or denuded (stripped) epithelium

	Non-linear regression (NONLIN)		*Scatchard analysis*		*Hill analysis*
	K_D (pM)	B_{max} ($\times 10^{-17}$ mol/section)	K_D (pM)	B_{max} ($\times 10^{-17}$ mol/section)	n*H*
Control	75.2 ± 15.7	41.1 ± 4.8	73.7	41.7 ± 5.8	0.91 ± 0.05
Stripped	69.1 ± 10.7	20.5 ± 1.9	79.7	22.6 ± 2.4	1.02 ± 0.15

Data are presented as mean ± s.e.mean.

samples of guinea-pig trachea. Removing these luminal surface cells did not significantly alter the density of I-CYP binding sites in tracheal smooth muscle ($P > 0.1$). As can be seen in Figure 5, the density of I-CYP binding sites was higher in tracheal smooth muscle than in other submucosal structures but was negligible in tracheal cartilage.

Discussion

The potency of histamine was approximately 4 fold greater in epithelium-denuded guinea-pig tracheal rings than in intact preparations. In contrast, the potencies of ACh, CCh and K^+, as determined from first concentration-effect curves, were similar in intact and rubbed tracheal rings. The removal of the epithelium from guinea-pig tracheal rings by gentle rubbing with a cotton wool swab, produced no damage to tracheal smooth muscle that was discernible under the light microscope. The fact that maximal responses to the contractile agonists ACh, CCh, histamine and K^+ were similar in control and epithelium-denuded preparations also suggests that the integrity of the smooth muscle was not compromised by this procedure. In contrast to this selective effect on histamine potency in the guinea-pig, removal of the epithelium from canine bronchi has recently been shown to enhance the potency of ACh, 5-hydroxytryptamine and histamine without altering E_{max} values (Flavahan *et al.*, 1985).

The apparently selective increase in sensitivity to histamine in epithelium-denuded tracheal rings of the guinea-pig may have been caused by a reduction in relaxant histamine H_2-receptor activity. However, results clearly show that the H_2-receptor antagonist cimetidine failed to alter the contractile potency of

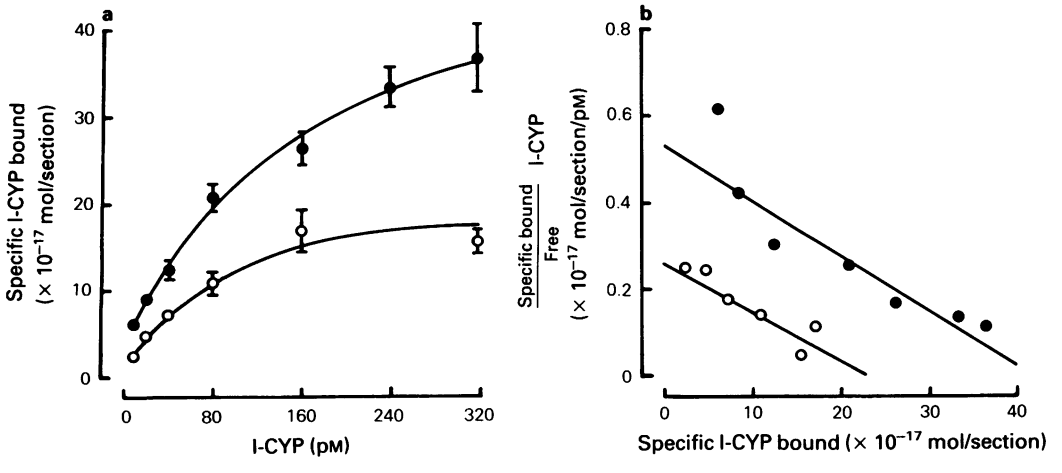

Figure 4 (a) Saturation of specific binding of [^{125}I]-iodocyanopindolol (I-CYP) in sections of guinea-pig trachea with an intact (●) epithelium and with the epithelium removed (○). (b) Scatchard analysis of the specific binding of I-CYP in guinea-pig tracheal sections with (●) and without (○) epithelium. Each point represents the mean of at least 6 observations. Vertical lines represent s.e.mean.

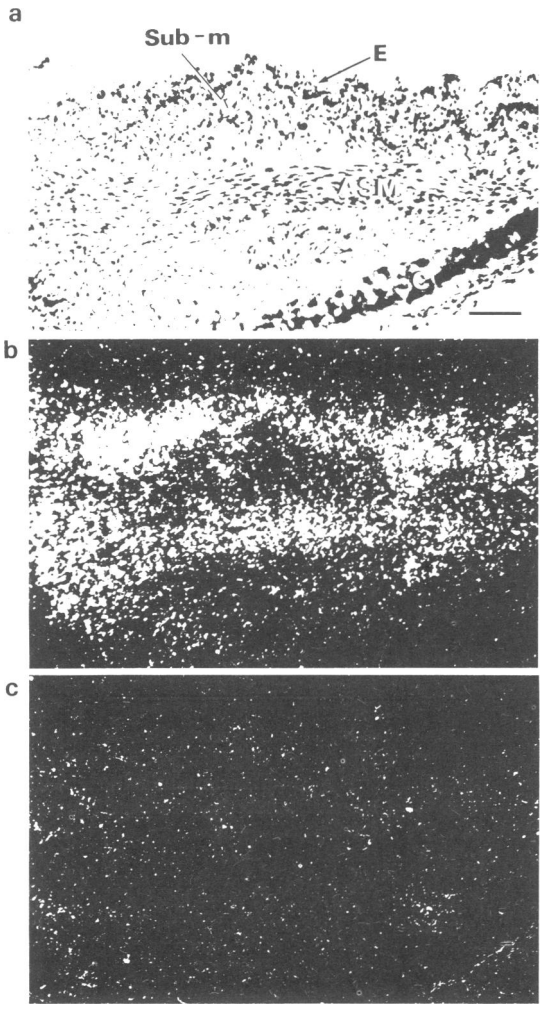

Figure 5 Distribution of [^{125}I]-iodocyanopindolol (I-CYP) binding sites in transverse frozen sections (10 μm) of guinea-pig trachea with an intact epithelium. (a) Lightfield photomicrograph showing epithelium (E), airway smooth muscle (ASM), submucosa (Sub-m) and cartilage (C). (b) Darkfield photomicrograph of the same section showing the distribution of autoradiographic grains following incubation with I-CYP. Grains are primarily localised over E and ASM with a lesser density over submucosal structures. Grain density over cartilage was no greater than background. (c) The subsequent serial section incubated with I-CYP in the presence of 200 μM (−)-isoprenaline shows no specific localisation of grains. Bar = 100 μm.

histamine in either stripped or intact rings. Furthermore, stripped preparations continued to be significantly more sensitive to histamine than control rings in the presence of cimetidine. This is consistent with data showing that the function of the H_2-receptor is of relatively minor significance in guinea-pig central and peripheral airways smooth muscle (Drazen *et al.*, 1978; Okpako *et al.*, 1978; Duncan *et al.*, 1980).

Alternatively, the guinea-pig tracheal epithelium may produce a relaxant factor in response to histamine which modulates the H_1-receptor-mediated contractile response of the smooth muscle. Endothelium-dependent relaxations of vascular smooth muscle in response to histamine, ACh and 5-hydroxytryptamine have recently been described (Furchgott, 1983; Vanhoutte & Rimele, 1982).

The maximal relaxant effects of the β-adrenoceptor agonist isoprenaline, the phosphodiesterase inhibitor theophylline and the adenyl cyclase stimulant forskolin (Seamon *et al.*, 1981) were significantly lower in stripped than in intact tracheal rings precontracted maximally with CCh. These data suggest that while tracheal relaxation to these agonists is not epithelium-dependent, it is modulated by an inhibitory signal generated by these cells. A similar effect on maximal responsiveness to isoprenaline was observed in canine bronchi (Flavahan *et al.*, 1985). However, the maximal response to nitroglycerin was not altered by epithelium stripping. Thus it may be that the inhibitory signal is induced in response to relaxants causing increases in intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels, such as isoprenaline, forskolin and theophylline, but not to those selectively generating guanosine 3′:5′-cyclic monophosphate (cyclic GMP) such as nitroglycerin (Diamond & Chu, 1983; Itoh *et al.*, 1985). In contrast, the relaxant potencies of each of these agonists were not significantly altered by removing the epithelium.

In the present study, the autoradiographic identification of tracheal binding sites for I-CYP clearly shows that a greater density of β-adrenoceptors existed on epithelial cells than on smooth muscle, which is consistent with previous findings in the rat (Xue *et al.*, 1985) and ferret lung (Barnes & Basbaum, 1983). While stimulation of such β-adrenoceptors is thought to increase the active transport of chloride ions across the tracheal epithelium (Davis *et al.*, 1979; Al-Bazzaz & Al-Awqati, 1979), as well as to increase the velocity of airway mucus transport (Mossberg *et al.*, 1976a,b), they may also mediate the generation of a smooth muscle relaxant factor. It has been suggested that the vasodilator effect of isoprenaline in canine coronary arteries is partly mediated via an endothelium-derived relaxant factor (Rubanyi & Vanhoutte, 1985). It seems likely that the reduced responsiveness of epithelium-denuded tracheal rings to isoprenaline resulted from the inability of these preparations to produce a relaxant factor, rather than the result of mechanical damage to smooth muscle causing a decrease in β-adrenoceptor function. Autoradiographic analysis has

shown that the density of I-CYP binding sites in smooth muscle was similar in intact and stripped preparations. Furthermore, removing the tracheal epithelium had no significant effect on the high affinity I-CYP binding to airway smooth muscle.

Disruption of the airway epithelium is known to occur in asthma and may be associated with bronchial hyperreactivity to airway spasmogens (Boushey *et al.*, 1980; Hogg & Eggleston, 1984). In addition, airway β-adrenoceptor dysfunction is known to occur in severe asthma (Paterson *et al.*, 1982; 1984). It is possible that the loss of bronchial epithelium exacerbates any loss of airway smooth muscle β-adrenoceptor function in asthma.

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